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Acquisition rate of antibody to hepatitis B surface antigen among medical and dental students in Japan after three-dose hepatitis B vaccination

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ABSTRACT

Background: Health care workers (HCWs) are at high risk of contracting blood-borne infections including hepatitis B virus (HBV) infection. In Japan, all HCWs are required to receive HB vaccination before beginning work. This study aimed to investigate the dynamics of the HB surface antibody (anti-HBs) titer after a three-dose HB vaccination in HCWs and to determine effective scheduling of HB vaccination for non-responders.

Methods: Subjects included 832 medical and dental students who had received a three-dose HB vaccination (Bimmugen® 0.5 ml/vial). Anti-HBs was measured three times (before the third dose and 1 and 5 months after the third dose) using the CLIA method. The cut-off value of anti-HBs acquisition was 10 mIU/mL. After booster doses (three maximum) were administered to non-responders, the anti-HBs titers were measured again.

Results: Out of 832 students, 491 were analyzed, of which 58.9% (289) were male. Anti-HBs-positive rates before the third dose and 1 and 5 months later were 47.9%, 95.9%, and 89.0%, respectively. The relationship between the antibody titer at one month (x) and 5 months (y) was estimated by $\log_{10} y = \log_{10} x - 0.134$ ($P < 0.0001$). Twelve non-responders were followed-up, all of which acquired a protective anti-HBs titer after revaccination with a three-dose booster.

Conclusion: Anti-HBs titer decreases by an average of 20% within 4 months between the 1st and 5th month after the third dose. Therefore, anti-HBs titer should be measured periodically after completing the three-dose vaccination. Additionally, results suggested that booster doses are effective if administered with the same schedule as primary vaccination.

1. Introduction

Hepatitis B virus (HBV) infection is a major public health concern worldwide. In 2015, the World Health Organization (WHO) reported that an estimated 257 million people were living with persistent HBV infection, among which 887,000 people died annually of its complications, including cirrhosis and hepatocellular carcinoma [1].

HBV has strong infectivity. According to studies in chimpanzees and chimeric mice inoculated with HBV, the viral load for the minimum 50% infectious dose was estimated at 10 copies [2,3]. The age at which a person becomes infected HBV is the factor whether acute or chronic infection occurs. 80–90% of infant infected during their first year of life and 30–50% of children infected before 6 year-old progress to chronic infection while only 5% of newly infected adult develops chronic infection [1]. Vaccination to HCWs is effective to prevent mainly acute hepatitis including fulminant HBV. It can also reduce occult HBV infection, which may result in

Abbreviations: ACIP, advisory committee on immunization practices; Anti-HBs, HB surface antibody; CDC, Centers for Disease Control and Prevention; HBV, hepatitis B virus; HCV, hepatitis C virus; HCWs, health care workers; HICPAC, Hospital Infection Control Practices Advisory Committee; HIV, human immunodeficiency virus; WHO, World Health Organization.

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fulminant hepatitis [4] when the host becomes immunosuppression.

The virus is transmitted through personal contact or accidental injury with infectious sharp materials [5]. The infectious route may differ based on the endemic level. The chance of horizontal infection increases in hyper endemic region. In developed countries, vertical transmission is efficiently prevented by effective screening and prompt intervention using immunoglobulin and birth-dose vaccination. The transmission route may have a strong association with horizontal infection through personal or sexual contact, and the high transmission risk is mainly observed in high-risk populations [6].

In addition to hepatitis C virus (HCV) and human immunodeficiency virus (HIV), HBV is a well-known occupational hazard to health care workers (HCWs) according to health care guidelines because of its high viability outside the body and infectiousness. The main infectious route for HCWs is percutaneous exposure to contaminated blood via accidental injury mediated by needles and other sharp objects; the associated transmission risk of HBV, HCV, and HIV is 37.6%, 39%, and 4.4%, respectively [7].

In 2002, WHO reported that 2 million out of a total of 35 million HCWs experience percutaneous exposure to infectious diseases each year. Therefore, prevention of HBV infection among HCWs has become a crucial issue. After introduction of HB vaccination in 1981, the Centers for Disease Control and Prevention (CDC) of the United States provided a platform for preventive measures among HCWs that responders to HB vaccination do not need immediate medication such as HBIG. [5]. In contrast to HCV, HIV, and other blood-borne infections such as syphilis and viral hemorrhagic fever, HTLV-1 etc, HBV can be prevented by effective vaccination. It was reported that 95% of vaccinated healthy infants, 92% of HCWs aged <40 years, and 84% of healthy HCWs aged ≥40 years could acquire protective antibody to HBV with a half-life of 15–30 years [8].

The Advisory Committee on Immunization Practices (ACIP) and the Hospital Infection Control Practices Advisory Committee (HICPAC) did not recommend a post-vaccination serology test after completion of the HB vaccination series in their 1997 guideline [9]. However, in 2013, the CDC mandated administration of the HB vaccine to all unvaccinated HCWs before entry to the medical care setting followed by a post-vaccination serology test 1–2 months after completion of the vaccination to determine whether further pre-exposure prophylaxis is necessary. [10]. Controversially, in the new WHO guideline for HBV published in 2017, the routine post-vaccination serology test was not recommended [11].

In Japan, although HBV prevalence among the general population and in children under 5 years old was very low at up to 0.201% and <0.1%, respectively, in 2011 [12], HCWs remain at risk of transmission. In the vaccine guidelines for medical personnel produced by the Japan Society of Infection Prevention and Control [13], it is strongly recommended that all medical personnel—including medical students—who are currently engaged or will be engaged in a health care job are actively immunized to preventable diseases, especially HB infection. However, this guideline did not recommend subsequent post-vaccination serology testing after confirming anti-HBs acquisition.

The aim of this study was to determine the HB surface antibody (anti-HBs) acquisition rate among medical and dental students in Hiroshima, Japan. After receiving the standard three-dose primary vaccination, non-responders among the study group were administered booster doses according to three post-vaccination serology tests of anti-HBs titer just before the third dose and 1 month and 5 months later. Then, the dynamics of the anti-HBs titer were evaluated. We addressed the current problems and investigated effective scheduling of HB vaccination of so-called non-responders who never gained the protective antibody titers after receiving the standard three-dose HB vaccination.

2. Methods

2.1. Subjects

We recruited a total of 832 medical and dental students of Hiroshima University who had received the standard three-dose HB vaccine subcutaneously according to the WHO-recommended schedule of 0, 1, and 6 months during the enrollment period from 2011 to 2016. The HB vaccine used in this study was Bimmugen®, which is a recombinant adsorbed HB vaccine derived from yeast and manufactured by the Chemo Sero Therapeutic Research Institute (Kaketsuken), Japan. Bimmugen® is prepared in two different forms—Bimmugen® 0.25 ml for infants and Bimmugen® 0.5 ml for young children and adults—and it can be administered intramuscularly or subcutaneously. This vaccine was approved for clinical use on March 29, 1988 [14].

To detect anti-HBs titers of all vaccinated students, we performed three post-vaccination serology tests just before the third dose and 1 month and 5 months later. Before the third HB vaccine dose, we obtained informed consent from each study subject by explaining the content of the study and confirming their willingness to participate in the study. We then proceeded with the post-vaccination serology test based on this study protocol. The students who had incomplete post-vaccination serology tests for any reason were excluded, and the remaining 491 students (289 males and 202 females) were the subjects of the final analysis.

2.2. Method

Anti-HBs titer was measured by the chemiluminescent immunoassay method (CLIA, Architect Osabu® Abbott Japan Co., Ltd.). The resultant anti-HBs titer was interpreted according to the following cut-offs:

(i) "negative" for anti-HBs < 10 mIU/mL
(ii) "positive" for 20 mIU > anti-HBs ≥ 10 mIU /mL
(iii) "strongly positive" for ≥ 20 mIU/mL

The anti-HBs titer of ≥10 mIU/mL is considered anti-HBs positive, irrespective of the protective strength.

We examined the trend of anti-HBs titer measured just before the third dose and the 1st month and 5th month thereafter. Regression analysis was performed to examine the relationship between anti-HBs titer 1 month and 5 months after the third HB vaccine dose. JMP10 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

We recommended that non-responders who were negative for anti-HBs throughout the three tests repeat the booster doses without exceeding a maximum of three doses (total of six doses including primary vaccination). We highly recommended that the non-responders who received three booster doses after their primary three-dose HB vaccination be revaccinated with the same schedule as the primary vaccination on a 0, 1, and 6-month basis as confirmed in this study. The post-vaccination serology testing was performed for those non-responders within 1 year after they received booster doses to determine the antibody acquisition.

Finally, we calculated the antibody acquisition rate based on the results of the standard three-dose HB vaccination and another three-dose booster vaccination of those who participated in and were followed-up until the end of the study.

2.3. Ethical considerations

All participants were well informed regarding the content of the study, and informed consent was obtained from each. This research was approved by the Epidemiological Ethics Review Committee of Hiroshima University (Hiroshima University Epi- 455).

3. Results

Fig. 1 shows a flowchart of subjects. Out of 832 students who were recruited, 491 (59.1%) students completed the study protocol, and their data were analyzed. We confirmed 17 subjects who could not acquire anti-HBs after 3-dose vaccination and tried to follow up for additional dose. We could follow up 12 subjects among them. Therefore, we could follow up 486 subjects in total during the survey. The transition of the anti-HBs titer of 491 subjects after standard three-dose HB vaccination just before, the 1st month and the 5th month are shown in Fig. 2. Among 491 students, 289 were male, and 202 were female. The mean age among 491 subjects were 22.7 ± 2.8. The anti-HBs-positive rate was 47.9% immediately before the third dose and rose to 95.9% in the 1st month and 89.0% in the 5th month. Immediately before the third dose, there was a significant difference in the anti-HBs-positive rate between males and females. Seventeen subjects (3.5%) did not acquire anti-HBs positivity according to anti-HBs testing just before the third dose and the 1st and 5th month after.

Fig. 3 shows the reduction rate of anti-HBs titer after the standard three-dose HB vaccination. Assuming the 1-month anti-HBs titer as X and the 5-month anti-HBs titer as Y, the relationship between the titers at the 1st month and the 5th month after completion of the three-dose HB vaccination was represented by the following formula: $\mathrm{Log}\ (y) = -0.134 + 0.87 \times \mathrm{Log}\ (x)$ $R^2 = 0.79$, $P < 0.0001$. The anti-HBs titer showed a nearly 20% decrease within the 4 month duration following the 1st anti-HBs testing.

The statuses following the booster doses and the respective antibody tests are shown in Fig. 4. Out of 17 students, five students were not able to be followed-up. Therefore, it was impossible to obtain their information related to booster doses and antibody tests. Twelve students followed the recommendation of Hiroshima University to receive the booster doses on the same schedule as the primary three-dose HB vaccine. Of those, 10 students received the Bimmugen® HB vaccine, but the brand name of the HB vaccine received by the other two students is unknown. Among the students, one received a single booster dose, seven received two booster doses, and four received three booster doses. After completing the booster dose administration, all students tested anti-HBs positive.

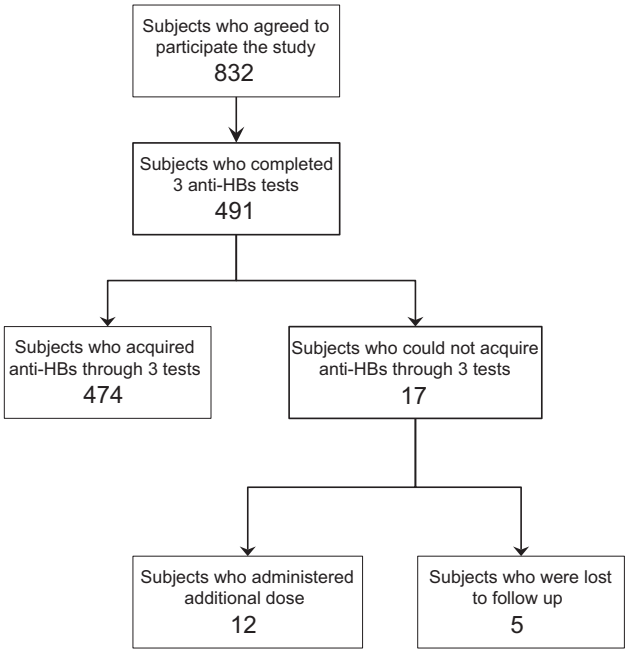


Fig. 1. Flowchart of study subjects. The flowchart shows the schedule of both the primary three-dose vaccination and the follow-up post-vaccination serology testing indicated by down sided black and white arrows, respectively.

Antibody acquisition through the standard three-dose HB vaccination and subsequent three-dose booster vaccination was tested—particularly for non-responders—in 832 students, 486 of which could be followed-up until the end of the study. The antibody acquisition rate of the 832 subjects after the standard three-dose HB vaccination was 95.2%, but this rate suddenly increased to 98.4% after subsequent booster dose administration. Furthermore, the antibody acquisition rate among the 486 students receiving the standard three-dose HB vaccination who were followed-up until the end of the survey was 97.5%; this rate was increased to 100% after administration of subsequent booster doses.

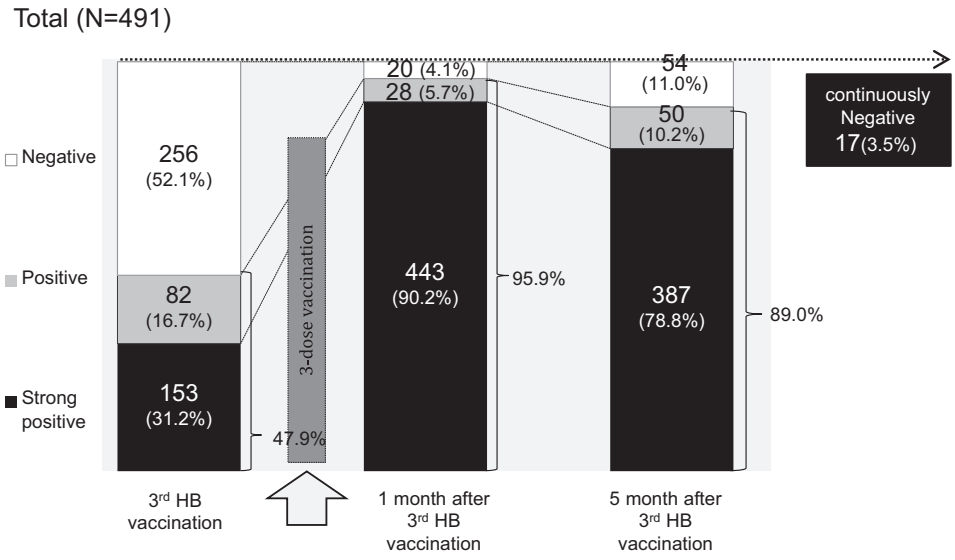
4. Discussion

Hepatitis B vaccination should cover all health care workers. But in this study, we selected the medical and dental students as our subjects because they are the future HCWs and have potential of dealing with blood. So, it is important to have protective anti-HBs titer before entering the hospital work as well as it is important to know the percentage of responders and non-responders after complete vaccination.

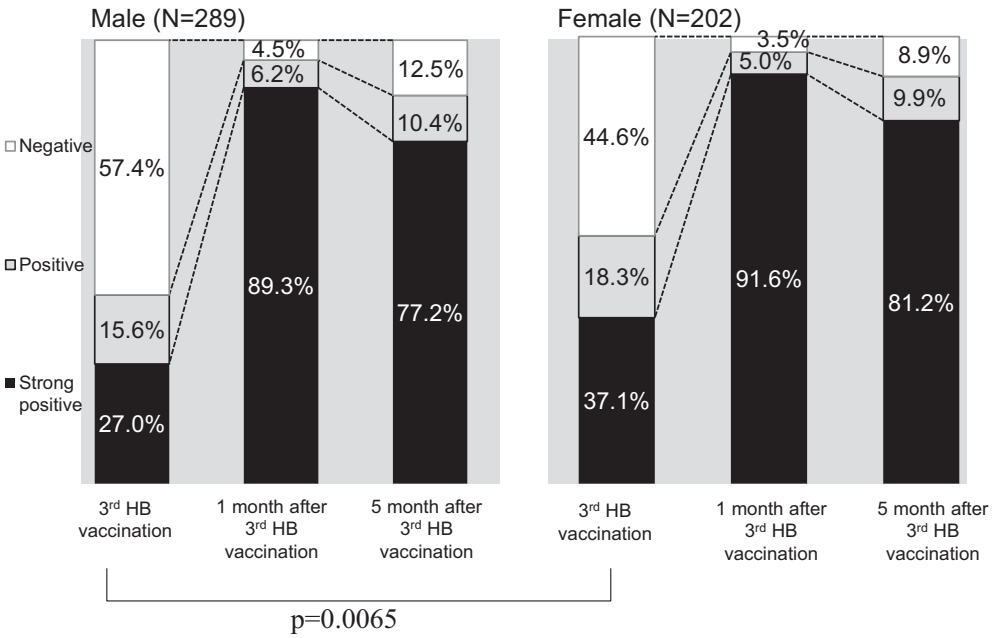
The principal finding of this study is the gradually increasing trend of anti-HBs acquisition from just before the third dose of standard three-dose HB vaccination to 1 month and 5 months after. Moreover, the dramatic decline in anti-HBs during the 4-month follow-up survey provided supportive evidence of the importance of periodic post-vaccination testing.

Anti-HBs titer induced by the HB vaccine gradually declines over time. Such decrease is observed in those who received the vaccine at an early or older age, especially at more than 40 years old [15]. As previously reported, 16% of persons vaccinated at an age <1 year had a detectable level of protective anti-HBs even after 18 years, whereas 74% of people vaccinated at an age ≥1 year had a detectable level. Another study showed that 92.9% of those who received the standard three-dose vaccine at a mean age of 14.5 years had detectable protective anti-HBs ≥10 mIU/mL after 23.2 years. These evidence-based findings suggested that the booster dose is not necessary for healthy HCWs who are less prone to infection [16,17]. The results of the linear regression analysis of the antibody titer in this study revealed that the anti-HBs titer decreased by approximately 20% within the 4-month duration following the 1st month of primary vaccination.

However, vaccination had no effect on production of anti-HBs for 5% of those study subjects who were either immunocompetent or immunosuppressed after receiving the primary three-dose HB vaccination. These non-responders could achieve seroconversion after either a single booster dose or three-dose revaccination. Health center of the university in Japan provided two boosters to every non-responder and checked for anti-HBs. If anti-HBs were still negative, health center provides one more booster and then checked anti-HBs again. The interval was within 1 year but it may vary depending on the student's schedule. Although the limitation still exists for the effectiveness of a high-dose single booster over the three doses vaccination [18], this study shows the



1 Transition of anti-HBs after HB vaccine in 491 adolescents



2 Transition of anti-HBs after HB vaccine in 491 adolescents

Fig. 2. Transition of anti-HBs after HB vaccination of 491 adolescents. (1) The trend of anti-HBs acquisition and its transition among 491 adolescents after completion of standard primary vaccination. Anti-HBs titers were detected just before the third dose and 1 and 5 months later. The white-colored box represents negativity, the gray-colored box represents positivity, and the black-colored box represents strong positivity of anti-HBs. (2) The trends identified in 289 male students and 202 female adolescents in a separate figure box. The white-colored box represents negativity, the gray-colored box represents positivity, and the black-colored box represents strong positivity of anti-HBs.

effectiveness of even a single booster dose. In 2013, the CDC (ACIP) recommended not to exceed more than three booster doses for non-responders (i.e., not exceeding a total of six doses) [10].

A previous study suggested that polymorphism of human leukocyte antigen (HLA) may be involved in the mechanism of low acquisition rate or non-response to the HB vaccine, indicating the possible efficacy of changing the type of HB vaccine used for the revaccination of those who had a low acquisition rate or non-responders [13]. Subjects with no antibody positivity after the standard three-dose HB vaccination became anti-HBs positive (70.6%) after receiving subsequent booster doses. We used the same kind of vaccine from the same company for those non-responders who needed boosters and it was found to be effective. So, we suggested that using the same brand for non-responders during the booster course is effective to gain protective anti-HBs. Additionally, one report has demonstrated an acquisition rate of 86.2–96.0% following booster dose administration after the three-dose vaccination [19]. This discrepancy supports the notion that there is a proper schedule of administration of additional doses. One year after the standard three-dose vaccination, additional booster doses were administered to those who could not acquire antibody despite following the same procedure for three-dose HB

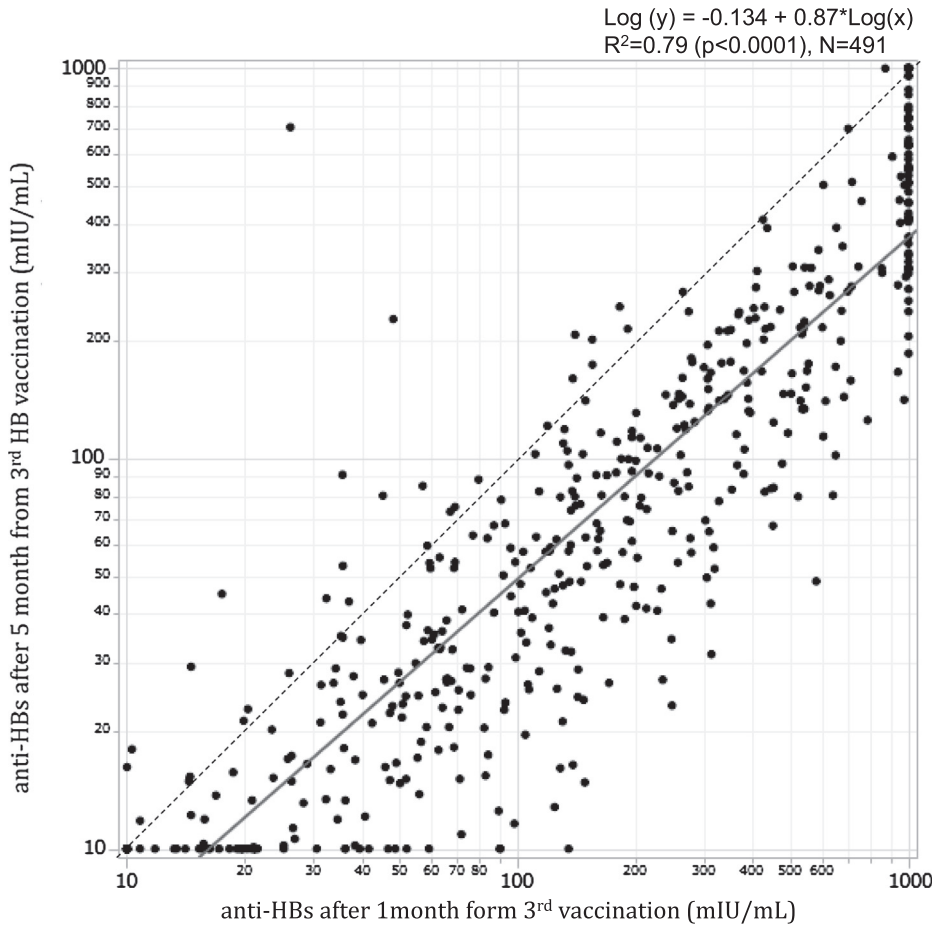


Fig. 3. Relation between amount of anti-HBs titer after 1 month of 3-dose HB vaccine and that after 5 months among 491 students. The anti-HBs titer measured 1 month after the third dose was plotted against that measured 5 months after the third dose. The horizontal line represents the anti-HBs titer at 1 month, and the vertical line represents the anti-HBs titer at 5 months after the third dose. The solid line represents the borderline margin, which indicates the linear relationship between the two variables as represented by Log (y) = −0.134 + 0.87 × Log (x) R^2 = 0.79.

vaccination. Additionally, this study suggested a successful schedule of revaccination of non-responders and strongly supported the use of with the same type of HB vaccine when additional booster doses are needed.

Now in the United States of America, new vaccine called Heplisav-B (Dynavax Technologies, Berkeley, CA), a yeast-derived vaccine prepared with a novel adjuvant, administered as a 2-dose series (0, 1 month) for use in persons aged ≥18 years had been produced and approved by CDC [20]. But in Japan, Heplisav-B has not been approved yet by Pharmaceutical and Medical Devices Agency of Japan (PMDA). In the future, this new vaccine may be introduced in Japan.

As previously reported [21], HCWs who frequently care for HBV-infected patients should maintain high antibody titer. In contrast to the general population, HCWs continuously face infection risks because they care for patients and handle infected medical instruments at their workplace on a daily basis. This is considered an occupational risk that may affect their lifespan. Specifically, HCWs are at high risk of contracting blood-borne infections, which can be transmitted percutaneously or via personal contact. Based on the guidelines of the CDC (immunization recommendations of ACIP and HICPAC) [16], it is considered that acute hepatitis can be effectively prevented by administering a booster to people, including medical personnel, who frequently face HBV infection risks [22].

A previous study suggested that when the anti-HBs titer reached or exceeded 10 mIU/mL, active immunity was sustained for 10–25 years [23]. Another study revealed that the percentage of HCWs whose anti-HBs titer decreased below the protective level 16–20 years after vaccination was 25% [15]. As the antibody titer decreases following acquisition, it is strongly recommended to test anti-HBs titer periodically to ensure proper maintenance of the antibody titer for effectiveness.

One major issue, which was addressed in the current study, is that some recipients do not respond to the three-dose HB vaccine. As indicated previously in some studies, nearly 5% of three-dose HB vaccine healthy recipients are non-responders [24,25]. In this study, 3.5% of students who did not acquire anti-HBs throughout the duration of anti-HBs testing were categorized as non-responders. Nonetheless, testing for anti-HBs is suggested to ensure proper protection.

This study had some limitations. Firstly, this study did not evaluate the possible causes of non-response to HB vaccination. Yoshioka et al. found that the durability of immunity depends on the primary anti-HBs titers [26]. However, the age at vaccination was found to be associated with anti-HBs acquisition [15]. Smoking habits and obesity were also possible risk factors for non-responders [27].

In conclusion, this study illustrated the necessity of post-vaccination testing among HCWs, which is consistent with the current ACIP recommendation regarding HB vaccination of HCWs. Additionally, revaccination with the standard three-dose vaccine must be performed on a proper schedule for non-responders to primary vaccination.

No	Gender	Age	Times of additional vaccinated	anti-HBs after additional vaccinated (mIU/ml)
1	Female	20	3	98.8
2	Male	21	3	69.9
3	Male	20	3	59.5
4	Male	25	3	52.6
5	Male	21	2	187.3
6	Female	25	2	61.5
7	Male	21	2	52.1
8	Female	21	2	51.6
9	Male	22	2	40.1
10	Female	23	2	36.7
11	Female	22	2	16.8
12	Male	23	1	185.0
13	Male	25	LTF	
14	Male	23	LTF	
15	Male	22	LTF	
16	Female	24	LTF	
17	Female	22	LTF	

※LTF : Loss to follow up

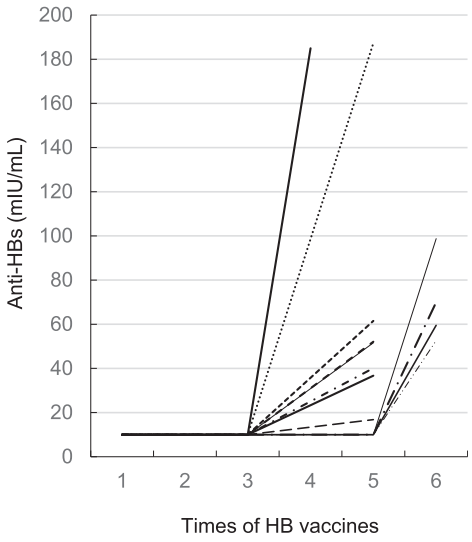


Fig. 4. Anti-HBs titer of 12 cases who received additional HB vaccine out of 17 cases kept Negative after 3-dose HB vaccine. The table (left) indicates the anti-HBs titer related to the total of 17 non-responders, and the graph (right) describes the pattern of anti-HBs titer after the booster doses were administered to 12 non-responders, who received at least one booster dose after primary vaccine failure. Each colored line represents the scenario of an individual non-responder.

Declaration of interest

The authors declared that there is no conflict of interest.

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Author contributions

SN wrote the manuscript with input from all authors. **SN**, **CY**, **KKo**, **CC**, **AS**, and **KKe** conducted the survey. **MO**, **TA**, **MY**, and **JT** played a key role data management, and calculation. **KKo, TA and JT** developed manuscript. **JT** conceived the study and was in charge of overall direction, planning and contributed to the final version of the manuscript.

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